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Review

# Ion channels and transporters in metastasis☆

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ABSTRACT

An elaborate interplay between ion channels and transporters, components of the cytoskeleton, adhesion molecules, and signaling cascades provides the basis for each major step of the metastatic cascade. Ion channels and transporters contribute to cell motility by letting through or transporting ions essential for local $Ca^{2+}$, pH and – in cooperation with water permeable aquaporins – volume homeostasis. Moreover, in addition to the actual ion transport they, or their auxiliary subunits, can display non-conducting activities. They can exert kinase activity in order to phosphorylate cytoskeletal constituents or their associates. They can become part of signaling processes by permeating $Ca^{2+}$, by generating local pH-nanodomains or by being final downstream effectors. A number of channels and transporters are found at focal adhesions, interacting directly or indirectly with proteins of the extracellular matrix, with integrins or with components of the cytoskeleton. We also include the role of aquaporins in cell motility. They drive the outgrowth of lamellipodia/invadopodia or control the number of β1 integrins in the plasma membrane.

The multitude of interacting ion channels and transporters (called transportome) including the associated signaling events holds great potential as therapeutic target(s) for anticancer agents that are aimed at preventing metastasis. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.



**Contents**



## 1. Introduction

Metastasis is based on a complex, multi-step process called the metastatic cascade [1]. In carcinomas, i.e. tumors developing from epithelial cells, the metastatic cascade typically begins with an epithelial–mesenchymal transition (EMT), another multi-step process, during which cells, originally featuring epithelial characteristics transmute into cells that display mesenchymal characteristics [2]. To acquire a mesenchymal phenotype, epithelial cells that are usually attached to a basement membrane undergo numerous biochemical and molecular changes such as the activation of transcription factors, the modified expression of specific cell-surface proteins, the expression and reorganization of cytoskeletal proteins, changes in microRNA expression and secretion of ECM (extracellular matrix) — degrading enzymes. These changes are accompanied by a loss of cell–cell contacts, an increase in cell motility (migration and invasiveness), a strengthened resistance to apoptosis, and an enhanced production of matrix components [2].

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During embryonic development, postnatal growth and wound healing, EMT occurs in an orderly way and is terminated by its reverse process called MET (mesenchymal–epithelial transition), whereas the acquisition of an invasive, eventually metastatic phenotype due to a delay or even a lack of a controlled MET represents EMT's pathological potential. EMT is accompanied by changes in the expression patterns of transcription factors such as SNAIL, TWIST or ZEBs which leads to a downregulated expression of E-cadherin, ZO1, claudins and occludins and an upregulated expression of N-cadherin, ECM components such as collagens and fibronectin, and matrix metalloproteases (MMPs) [3]. It is conceivable that ion channels and transporters are involved in EMT since their expression is as well modulated by transcription factors. For instance, the unscheduled expression of a neonatal splice variant of the voltage gated sodium channel $Na_v1.5$ ($nNa_v1.5$) clearly correlates with migration and invasion of metastatic breast cancer [4–6].

In general, a malfunctioning or oncogenic EMT can lead to metastable cellular phenotypes combining both epithelial and mesenchymal characteristics [7]. EMT includes loss of contact inhibition and the cells' ability to break out of the organized tissue structure [8–10]. The following detachment from the primary tumor requires the release of intercellular junctions that are typically mediated by cadherins or integrins [11,12]. The next step in the metastatic cascade is the cellular invasion of the surrounding stroma. For this purpose cells remodel the extracellular matrix by secreting matrix metalloproteinases and simultaneously exhibit a dynamic cell–substrate interaction in order to migrate directionally [13]. The migrating cells eventually enter the vasculature or the lymphatic system where they become circulating tumor cells as they are being carried away by the blood or lymph stream. Only a very small percentage of these circulating tumor cells survive the extracellular milieu and adhere to the endothelium at a distant site [14]. The cells then extravasate and invade the surrounding tissue in order to form a metastasis which finally develops a full-grown secondary tumor [15]. All four steps of the metastatic cascade, (i) loss of cell–cell contacts, (ii) invasion of the surrounding stroma and the vasculature, (iii) adhesion to the endothelium, and (iv) extravasation into the tissue of the target organ require the presence and/or concerted action of ion channels and/or transporters (Fig. 1, Table 1), also referred to as migration-associated transportome [16].

## 2. EMT and loss of cell–cell contacts

Oncogenic epithelial–mesenchymal transition (EMT) is based on a coordinated gene expression program, includes the early steps of malignant transformation and is accompanied by a gain of pro-metastatic properties such as an increased basal motility including invasion and cancer stem cell characteristics [17–19]. A loss of cell–cell contacts can be induced by ectopic expression of carbonic anhydrase IX (CAIX) which results in the redistribution of CAIX and its pH-regulatory interaction partners, the $Na^+$,$HCO_3^-$ cotransporter NBC and the anion exchanger AE2, to the leading edge where they can fulfil tasks required for cell migration and invasion [20]. Thus, several ion transport proteins are involved in this process of epithelial–mesenchymal transition (EMT). Whether or not and to what extent other transport proteins associated with tumor metabolism, such as the $Na^+/H^+$ exchanger NHE1 or, monocarboxylate transporters (MCTs), or aquaporins contribute to EMT remains to be elucidated.

A $Cl^-$ and $HCO_3^-$ conducting channel, known as the cystic fibrosis transmembrane conductance regulator (CFTR), plays a role as an EMT suppressor in the human breast adenocarcinoma cell lines MCF-7 and MDA-MB-231 [21]. Both the presence and the function of CFTR decrease the metastatic potential of MCF-7 cells. Conversely, CFTR knockdown reduces the strength of cell–cell contacts by decreasing the expression of E-cadherin and occludins and – like the functional inhibition with inh172 or GlyH101 – leads to a dramatic increase in migration and invasion. On the same lines, the physical interaction of CFTR with the scaffolding protein NHERF1 ($Na^+/H^+$ exchanger regulatory factor1) in a CFTR–NHERF1–ezrin–actin multiprotein complex stabilizes cell–cell junctions through the tight-junction protein ZO-1 [22]. In nude mice, the growth of implanted MDA-MB-231 cells that overexpress CFTR is not reduced while the metastatic potential is clearly decreased, and in clinical breast cancer samples a low CFTR expression correlates with disease progression and poor prognosis [21]. Consistently, CFTR overexpression suppresses EMT and invasive behavior in MDA-MB-231 cells. A functional CFTR has been shown to control NFκB-mediated inflammatory signaling [23,24]. Knocking down CFTR leads to activation of NFκB, while conversely, the inhibition of NFκB countermands CFTR knockdown-induced EMT and the development of an invasive phenotype in MCF-7 cells. This indicates that CFTR acts as a tumor suppressor by tightly controlling NFκB-signaling via its ion-conducting function [21]. Studies employing a structurally functioning but at the same time transport-deficient CFTR could help clarify as to what extent purely structural protein interactions determine cell–cell adhesion and impact on malignancy. Interestingly, in colon cancer cells, CFTR and the adherens junction molecule AF-6/afadin physically interact at cell–cell contacts. When CFTR is knocked down these cells display an enhanced invasive phenotype which can be completely reversed by either AF-6/afadin overexpression or inhibition of ERK, indicating the involvement of the AF-6/MAPK pathway [25].

Calcium signaling mediated by calcium-permeable ion channels including the transient receptor potential-melastatin-like 7 (TRPM7) channel contributes to EMT in breast cancer, too [26]. Chelation of intracellular $Ca^{2+}$ by BAPTA or EGTA reduces EGF- and hypoxia-induced EMT and inhibits the EGF-dependent activation of signal transducer and activator of transcription 3 (STAT3), probably including CaMK/NFκB signaling [27], while leaving the Akt and ERK1/2 pathways entirely unaffected. Since silencing TRPM7 inhibits only parts of the EGF-induced processes inhibited by $Ca^{2+}$ chelation, additional $Ca^{2+}$ channels or -transporters other than TRPM7 must be involved [26].

The $Ca^{2+}$-activated $K_{Ca}3.1$ channel (KCNN4, IK1) participates in the EMT of colorectal cancer [28]. Stimulation of $K_{Ca}3.1$ expression by phosphatase of regenerating liver-3 (PRL-3) is accompanied by an elevation of the cytosolic $Ca^{2+}$-concentration $[Ca^{2+}]_i$ through an unknown mechanism, possibly through a constitutive entry through voltage-independent $Ca^{2+}$-permeable channels under hyperpolarization of the membrane potential. This increase in $[Ca^{2+}]_i$ causes a constitutive autophosphorylation of CaM-kinase II leading to phosphorylation of GSK-3β [29]. In human colon adenocarcinoma cells (LoVo) transfected with PRL-3, the expression levels of phosphorylated GSK-3β and the transcription factor SNAIL are increased. The increased expression of these proteins can be repressed by both $K_{Ca}3.1$ siRNA and the specific channel blocker TRAM-34 [28]. SNAIL inhibits E-cadherin expression not only in colorectal cancer [28] but also in breast cancer cells [30] promoting the loss of cell–cell contacts characteristic of EMT [3].

EMT is accompanied by a loss of cell contact inhibition. NIH3T3-cells stably transfected with $K_v11.1$ (hERG1) channels show a loss of cell contact inhibition. In culture, these cells grow in multiple layers and at high density [31]. Interestingly, their morphology changes from fibroblast-like to spindle-shaped while both the degree of cell polarization and migration increase. Furthermore, allogeneic transplantation of hERG1-expressing cells into nude mice leads to an increased incidence of tumors [31].

## 3. Invading the surrounding stroma and vasculature

In addition to being involved in EMT TRPM7 contributes to a more migratory and invasive phenotype [32], so that in human breast cancers, the expression level of TRPM7 and the formation of metastases are positively correlated. Knocking down TRPM7 leads to elevated myosin light chain (MLC) — and paxillin phosphorylation including an increased number of focal adhesions [33]. The resulting increases in contractility and adhesion strength cause a significant decrease in cell motility and thus metastasis. In this process TRPM7 functions via a “dual mode of

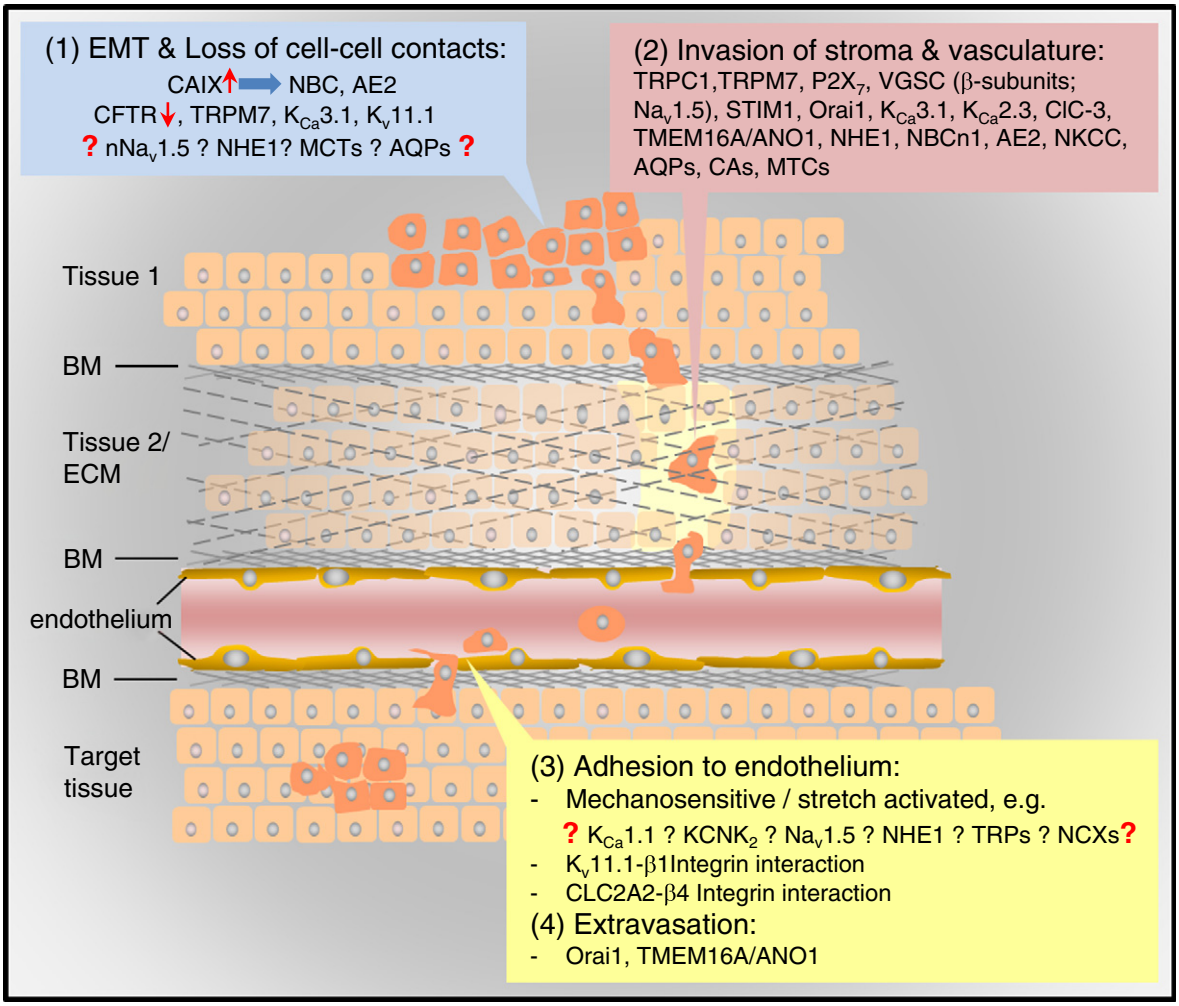


**Fig. 1.** Different steps of the metastatic cascade and the ion channels and transporters involved. (1) EMT can be induced by ectopic expression of CAIX leading to a redistribution of NBCs and the anion exchanger AE2 which enables migration. Downregulation of CFTR leads to a loss of cell–cell contacts. $Ca^{2+}$ signaling mediated by TRPM7 accompanies the process of EMT. An increase in $K_{Ca}$3.1 expression causes a loss of cell–cell contacts and transfection with $K_v$11.1 induces a loss of contact inhibition. The involvement of $Na_v$1.5, NHE1, MCTs and AQPs is possible and conceivable but so far has not been shown explicitly. (2) Numerous ion channels and transporters have been shown to contribute to cell motility and invasion. The transported ions ($Na^+$ through NHE1, NKCC, VGSC; $K^+$ through $K_{Ca}$3.1, $K_{Ca}$2.3, NKCC; $Cl^-$ through AE2, ClC-3, TMEM16A/ANO1, NKCC) trigger the movement of water through AQPs so that local osmotic swelling and shrinkage facilitate cell movement across a dense meshwork of ECM fibers. $Ca^{2+}$ entry (through TRPC1, TRPM7, P2$X_7$, STIM1, Orai1) stimulates the activities of kinases and $Ca^{2+}$ activated channels (e.g. $K_{Ca}$3.1, $K_{Ca}$2.3), and $Ca^{2+}$ signaling impacts on cytoskeletal dynamics. pH nanodomains controlled by NHE1, NBCn1, AEs and CAs modulate cytoskeletal dynamics, adhesion and intra- and extracellular enzyme activities including MMP and cathepsin. In addition to promoting NHE1 activity and cathepsin release, β-subunits of $Na_v$1.5 can serve as adhesion molecules. (3) In leukemia, adhesion to the endothelium could be facilitated by the interaction between $K_v$11.1 and β1 integrin. In circulating tumor cells, CLCA2 located at the endothelial surface can catch tumor cells by binding to the tumor cell's β4 integrin. Whether or not the shear stress in the vessel causes mechanosensitive channels and transporters such as $K_{Ca}$1.1, $KCNK_2$, $Na_v$1.5, NHE1, members of the TRP and NCX families or others to contribute to adhesion and subsequent diapedesis can only be speculated. (4) Extravasation of nasopharyngeal cells requires the $Ca^{2+}$ release-activated calcium channel protein 1 (Orai1). The step of diapedesis could also be facilitated by the $Ca^{2+}$-dependent activation of TMEM16A/ANO1. The resulting $Cl^-$ efflux would support local shrinkage of the evading cell and thus help the cell to squeeze its way into the target tissue. Please see text and Table 1 for further details and references. BM = basement membrane, ECM = extracellular matrix.

action" [34]. Its channel properties result in very temporary and local increases of $[Ca^{2+}]_i$, called $Ca^{2+}$ flickers [35], while its kinase activity induces the phosphorylation of myosin IIA heavy chain and eventually the transformation of focal adhesion sites into podosomes [36].

Similarly, independently of the actual ion translocation, the β subunits of voltage-gated sodium channels (VGSCs) exhibit a variety of functions contributing to the malignancy of breast cancer cells. They mediate tumor growth and promote invasion by facilitating the outgrowth of protrusions [37]. The β subunits are involved also in channel gating, and even in the absence of the pore-forming α subunits, they function as cell adhesion molecules [38]. The β1 subunits contribute to cellular aggregation and ankyrin-recruitment to cell–cell contacts by trans-homophilic adhesion [39,40]. In addition, they can interact heterophilically with the extracellular matrix protein tenascin-R [41], with β2 subunits [42], with N-Cadherin [43], NrCAM [42], contactin [44] and neurofascins [45].

### 3.1. The role of $Ca^{2+}$

In general, $[Ca^{2+}]_i$ has a major effect on the cellular migration machinery. Many constituents of the cytoskeletal migration machinery such as the above-mentioned myosin-light chain kinase (MLCK) [46], myosin II [47,48], calpain [49], $Ca^{2+}$ calmodulin-dependent protein kinase II [50,51] and the focal adhesion kinase [52] are $Ca^{2+}$ sensitive. $[Ca^{2+}]_i$ generates a myosin II-dependent contractile force leading to the retraction of the rear part of a migrating cell [53]. Strikingly, tiny, local $Ca^{2+}$ pulses occurring at the cell front only a few micrometers behind the leading edge trigger the withdrawal of defined areas of the lamellipodium by activating MLCK and myosin II-mediated contraction [46]. Fittingly, STIM1, a constituent of the store-operated $Ca^{2+}$ entry (SOCE) channel, is found close to the leading edge of endothelial cells [54] and pancreatic ductal adenocarcinoma (PDAC) cells [55]. Another component responding to $[Ca^{2+}]_i$ is the actin-binding protein α-actinin

**Table 1**
Ion channels and transporters in different steps of the metastatic cascade.

| Step | Ion channel/transporter and its mode of action | Reference |
|---|---|---|
| EMT | CAIX ⇒ ectopic expression induces loss of cell–cell contacts and redistribution of NBC and AE2 to the leading edge | [20] |
| | CFTR ⇒ EMT suppressor | |
| | – In breast cancer, controlling NFκB signaling, via its ion-conducting function | [21] |
| | – Through structural interaction at tight and adherens junctions | [22,25] |
| | TRPM7 ⇒ contributes to $Ca^{2+}$ signaling leading to EMT | [26,27] |
| | $K_{Ca}3.1$ ⇒ increased expression contributes to EMT in colorectal cancer | [28] |
| | $K_v11.1$ ⇒ loss of cell contact inhibition | [31] |
| Invading stroma & vasculature | TRPM7 ⇒ increase in cytosolic $Ca^{2+}$ & kinase activity = > podosome formation | [32–36] |
| | TRPM8 ⇒ contributes to invasiveness of oral squamous carcinoma via $Ca^{2+}$ | [141] |
| | TRPV2 ⇒ promotes prostate cancer cell migration | [142] |
| | STIM1 (constituent of SOCE) ⇒ polarized distribution in migrating PDAC | [54,55] |
| | – local $[Ca2^{+}]_i$ increases affect cytoskeletal dynamics & kinase/channel activity. | |
| | TRPC1 ⇒ local $[Ca2^{+}]_i$ affects cytoskeletal dynamics & kinase/channel activity | [61] |
| | $K_{Ca}3.1$ ⇒ local osmotic effects in glioma cells facilitate invasion | [58–60,62] |
| | $K_{Ca}2.3$ + Orai1 ⇒ breast cancer cell migration and bone metastases | [63,70] |
| | ClC-3 ⇒ local osmotic effects in glioma cells facilitate invasion | [50,58–61] |
| | AQP2 ⇒ supports migration by facilitating integrin turnover | [95] |
| | AQP3 ⇒ migration of breast cancer cells | [83] |
| | AQP4 ⇒ migration and invasion of glioma cells | [86] |
| | AQP5 ⇒ proliferation and migration of gastric cancer cells | [88] |
| | AQP8 ⇒ increases invasive potential of cervical cancer cells | [89] |
| | NHE1 ⇒ promotes motility and invasion by | |
| | – Osmotic effects | [75] |
| | – Generation of local $pH_e/pH_i$ environments at focal adhesions | [101,106–108] |
| | – Contributes to migration of melanoma, breast cancer, cervical cancer cells | [101–105] |
| | – Stimulates focal matrix digestion by local acidification | [133–135] |
| | AEs/AE2 ⇒ involved in cell motility by osmotic effects and pH regulation | [77,119] |
| | – Contributes to malignancy in hepatocellular carcinoma, gastric cancer and colonic carcinogenesis | [123–125] [127,128] [126] |
| | NBCn1 ⇒ involved in pH homeostasis of malignant breast cancer cells | [118,121] |
| | NKCC ⇒ osmotic effects | [78] |
| | VGSC (voltage gated sodium channels) ⇒ β subunits | |
| | – Support outgrowth of protrusions | [37] |
| | – Involved in channel gating, function as adhesion molecules | [38] |
| | – β1 subunits mediate cell–cell contacts via trans-homophilic adhesions and heterophilic interaction with matrix and cell surface-proteins | [39–45] |
| | $Na_v1.5$ ⇒ elevates metastatic potential of breast cancer by increasing | |
| | – NHE1 activity | [138] |
| | – Cysteine cathepsin activity | [139] [140] |
| | $P2X_7$ ⇒ increases breast cancer invasion by $Ca^{2+}$ depend. cathepsin release | [144] |
| Adhesion diapedesis | Kv11.1 ⇒ interacts physically and functionally with β1 integrin in leukemia | [149–152] |
| | – Activated by integrin binding, activates FAK activity | [152,153] |
| | CLCA2 ⇒ located in endothelia, interacts with β4 integrins on tumor cells | [155] |
| | Orai1 ⇒ required for extravasation of nasopharyngeal cancer cells | [156] |
| | TMEM16A/ANO ⇒ could facilitate diapedesis by local shrinkage | [68] |
| | Response to shear stress?? *Mechanosensitive* channels and transporters: $K_{Ca}1.1$, $KCNK_2$, $Na_v1.5$, NHE1, members of TRP and NCX families, others?? | ? |

that controls lamellipodial dynamics and directional migration [56]. Its actin bundling activity is inhibited by an increase in $[Ca^{2+}]_i$ [57]. An increase in $[Ca^{2+}]_i$ is also needed for the simultaneous activation of the $K^+$ channel $K_{Ca}3.1$ and the CaMKII-regulated $Cl^-$ channel ClC-3 [58]. Both channels contribute to glioma malignancy by promoting migration and invasion [59,60]. The required $Ca^{2+}$ entry is at least partially mediated by stretch activated TRPC1 channels (transient receptor potential cation channel 1) co-localizing with ClC-3 in lipid rafts of glioma cell processes since knocking down TRPC1 expression with shRNA leads to a decrease in $Cl^-$ currents in a $Ca^{2+}$ dependent way [61]. In addition, $Ca^{2+}$ release from intracellular stores, frequently induced by growth factor signaling and mediated by IP3, contributes to the required increase in $[Ca^{2+}]_i$. The $Ca^{2+}$ induced activation of $K_{Ca}3.1$ [62], $K_{Ca}2.3$ [63], ClC-3 [50,61] or the $Cl^-$ conducting TMEM16A/ANO1 [64–66], leads to a simultaneous efflux of $K^+$ and $Cl^-$ ions and eventually to a local, osmotic cell shrinkage [59,67,68], facilitating not only the global, $Ca^{2+}$-dependent retraction of the rear end of a migrating cell but also the fine-tuned process of squeezing through constrictions of the ECM meshwork [69].

A constitutive $Ca^{2+}$ entry promoting cancer cell migration and bone metastasis can also be accomplished by an intriguingly synergistic interaction between the voltage-independent $Ca^{2+}$ channel Orai1 and the small conductance $Ca^{2+}$ activated potassium channel $K_{Ca}2.3$ inside lipid rafts [70]. Treatment with the alkyl–lipid Ohmline disperses the lipid rafts and the $K_{Ca}2.3$–Orai1 complex, resulting in an impairment of the $K_{Ca}2.3$-dependent $Ca^{2+}$ entry, migration and metastasis.

### 3.2. Cell volume dynamics

Cells migrating on a substrate perform a repetitive cycle of protrusion of the lamellipodium and retraction of the rear part. Instead of lamellipodia, invasive cells develop functionally similar invadopodia [71] to explore and invade the ECM. Both the outgrowth of lamellipodia or invadopodia and the retraction of the rear end require local swelling and shrinkage, respectively [72–74]. These osmotic events are based on (i) local ion transport through the $Na^+/H^+$ exchanger NHE1 [75], the $Na^+$,$HCO_3^-$ cotransporter [76], the $Cl^-/HCO_3^-$ exchanger AE [77], and the $Na^+$, $K^+$, $2Cl^-$ cotransporter NKCC [78] at the leading edge/tip of

the outgrowing lamellipodium/invadopodium, (ii) local ion efflux across the above-mentioned $K^+$ and $Cl^-$ channels, and (iii) water molecules concomitantly passing through aquaporins [79] such as AQP1 [80], AQP3 [81–83], AQP4 [84–86], AQP5 [87,88], AQP8 [89], and AQP9 [90]. In human breast cancer (MDA-MB-231) and murine sarcoma (S180) cells, repetitive cycles of local, NHE1-dependent swelling at the cell front and shrinkage at its rear enable the cells to move independently of actin polymerization, myosin II, and integrin-mediated adhesion [91]. This may be the central mechanism underlying the rounded/amoeboid tumor cell migration mode that allows cell movement through a dense extracellular matrix without matrix degradation [92–94].

An intriguing function of AQPs has been shown for AQP9 [90]. AQP9 accumulates at certain sites in the plasma membrane. The resulting rapid, local water influx generates a hydrostatic pressure detaching the membrane from the cytoskeleton and pushing the membrane outwards. Previously membrane-anchored barbed end actin is now exposed and promotes actin polymerization in cooperation with actin monomers rapidly diffusing into the less viscous cytoplasm. This concerted action between local swelling and actin-polymerization controls the outgrowth of lamellipodia, filopodia or invadopodia.

Besides the permeation of water molecules a second mechanism by which AQPs act on cell motility has been shown for kidney epithelial cells [95]. AQP2 modulates the intracellular trafficking and subsequent cell surface presentation of the adhesion molecule integrin β1 by displaying a potential RGD (Arg-Gly-Asp) motif in its second external loop. These RGD motifs are generally present in ECM proteins (e.g. fibronectin, vitronectin) and bound by integrins in order to attach the cell surface to the ECM. Wild-type AQP2 mediates the internalization of β1 integrin and thus prevents its accumulation at the cell surface. This process supports cell migration by facilitating the turnover of integrins as parts of focal adhesions. The expression of an AQP2 RGD/A mutant or the absence of AQP2 leads to a considerable accumulation of β1 integrin in the plasma membrane and a reduction in motility.

### 3.3. pH modulates cell adhesion and actin dynamics

Depending on the composition of their subunits integrins interact with different proteins of the ECM. Various integrin dimers including $\alpha_2\beta_1$, $\alpha_5\beta_1$ and $\alpha_v\beta_3$ are sensitive to pH [96–98]. Hence, acidic extracellular pH values induce conformational changes of the integrin molecules leading to an enhanced avidity of the integrin headpieces to ECM proteins [98], or they modulate the mechanical stability of focal adhesions [99]. Since integrins protrude merely 20 nm into the extracellular space [100], rather the pericellular pH inside the glycocalyx than the global extracellular pH of the surrounding bulk solution modulates adhesion [96,101]. The pericellular pH is maintained by the proton-expelling activity of NHE1. NHE1 appears to play a major role in many tumors [102–104]. It is required for the motility of breast cancer, cervix carcinoma and melanoma cells [105]. In migrating melanoma cells, most of the NHE1 molecules accumulate at the leading edge where they are constituents of focal adhesions [106]. Consequently, the pericellular pH is more acidic at the cell front than at its rear end [101,107], and the most acidic pericellular pH nanodomains are restricted to focal adhesions at the cell front. These observations support the idea that NHE1 activity locally stabilizes the β1 integrin-mediated interaction between the cell surface and the ECM [106]. Not only in melanoma [108] but also in endothelial cells [109] the pericellular pH gradient is mirrored by a complementary intracellular pH ($pH_i$) gradient with $pH_i$ being more alkaline at the cell front than at the cell rear. The NHE1-dependent alkaline $pH_i$ at the cell front is thought to facilitate focal adhesion turnover due to a lower affinity of talin for binding actin [110]. The alkaline $pH_i$ stimulates also cofilin-regulated cell motility [111]. The alkaline $pH_i$ abrogates the blockade of cofilin activity by PI-(4,5)–P2-binding [112]. Cofilin then severs actin filaments and creates free barbed end actin. It thus establishes new sites of actin filament assembly and fuels dynamic actin polymerization that accompanies membrane protrusion at the cell front or at the tip of invadopodia [113]. The interaction between cofilin and cortactin depends on $pH_i$ as well. The NHE1-mediated increase in $pH_i$ causes the release of cortactin-bound cofilin which enables cofilin to generate free barbed actin, eventually promoting the polymerization of actin [114]. The more acidic $pH_i$ at the rear end activates the actin-binding protein gelsolin that regulates actin assembly and disassembly [115], and a neutral or slightly acidic $pH_i$ fosters the self-assembly of actin and the binding of myosin to actin [116,117].

In summary, the activity of pH regulating transporters such as NHE1 [103] and NBCs [118] or AEs [119], frequently in cooperation with CAs [20] and MCTs [120], creates the optimal $pH_i$ environment that drives the actin dynamics required for the outgrowth of lamellipodia/invadopodia.

NHE1 does not always play an exclusive, predominant role in the pH-regulation of cancer cells. In human breast cancer carcinomas, for instance, the expression of NBCn1 is twofold higher compared to normal breast cancer tissue obtained from the same patient [118,121]. This is accompanied by an increase in the clearly NBCn1-mediated cellular net-acid extrusion (=$HCO_3^-$ import) leading to an alkaline shift in the steady-state $pH_i$ by ~0.3 pH units.

While NHE1 and NBCn1 compensate for cellular acidosis or cause an increase in $pH_i$ by extruding protons or importing $HCO_3^-$, AE2 activity is usually triggered by an intracellular alkalinization and then extrudes $HCO_3^-$ [122]. In human hepatocellular carcinoma AE2 is overexpressed [123] and its inhibition induces apoptosis [124,125]. Induction of AE2 also contributes to colonic carcinogenesis through the activation of the extracellular signal-regulated kinase (ERK) pathway which can be blocked by AE2-targeted small interfering RNA (siRNA) or gastrin treatment [126]. In gastric cancer cells, however, AE2, downregulation of AE2 can be correlated with carcinogenesis which can be blocked by gastrin [127]. Gastrin stimulates the expression of AE2 in GC cells via early growth response 1 (EGR1), in a cholecystokinin B receptor (CCKBR) dependent manner [128].

### 3.4. Matrix metalloproteases (MMPs) and cathepsins clear the way

The outgrowth of invadopodia into the cell's surroundings requires enough space usually generated by degrading or remodeling the ECM [129]. Invadopodia secrete soluble enzymes with gelatinolytic activity such as cathepsins B (and others) [130] and the matrix metalloproteases MMP2 and MMP9 [131], or they display membrane-associated enzymes such as MT1-MMP (MMP14) [132] in order to degrade the surrounding ECM barrier. The action of several MMPs depends on the local, NHE1-mediated acidification at the cell surface of invadopodia [107,133–135]: The protonation of the ECM-component fibrinogen is essential for the MMP2 activity [136], and an acidic extracellular pH upregulates the expression of MMP9 [137]. Voltage-gated $Na^+$ channels ($Na_v1.5$) elevate the invasive potential of human breast cancer cells [138] by increasing the activities of NHE1 [139] and cysteine cathepsins [140]. The n-3 polyunsaturated docosahexaenoic acid (DHA), a natural ligand of the peroxisome proliferator-activated receptor β (PPARβ), inhibits $Na_v1.5$ expression reducing both the $Na^+$ current and NHE1 activity. Accordingly, DHA has no effect in cancer cells whose PPARβ expression is knocked down [4]. MMP activity can also be linked to $Ca^{2+}$ signaling [141,142]. Since the upregulation of MMP9 demands $Ca^{2+}$ influx, it can be antagonized by inhibiting voltage-gated $Ca^{2+}$ channels [143]. Interestingly, in the highly metastatic, human breast cancer cell line MDA-MB-435s the activity of the ATP-gated $Ca^{2+}$-permeable $P2X_7$ receptor increases invasion by the release of gelatinolytic cysteine cathepsins [144].

## 4. Adhesion to the endothelium and extravasation

Once they arrive in the blood or lymph stream, only 0.01% out of the circulating tumor cells will eventually form metastases [15], because of

either immune surveillance or mechanical stress caused by the blood flow [145,146]. Several strategies help the cells to survive this mechanical stress. They express high levels of stress proteins, e.g. the heat shock protein HSP70, or they interact with fibrinogen or platelets to form clots protecting them against immune cells and facilitating their arrest and their adhesion to the endothelium [147,148]. At this point, it remains highly speculative as to what extent ion channels and transporters contribute to the process of tumor cell adhesion to endothelial cells under shear stress conditions. Numerous ion channels and transporters such as $K_{Ca}1.1$, $KCNK_2$, $Na_v1.5$, NHE1 or members of the TRP and the $Na^+/Ca^{2+}$ (NCX) exchanger families are mechanosensitive and can be activated by stretching the plasma membrane. It is imaginable that shear stress could trigger their opening/activity or induce signaling mediated by their mechanosensitive domains. Thus, shear stress could induce pathways in tumor cells that induce the activation of integrins and might eventually lead to adhesion of tumor cells to endothelial cells. $K_v11.1$ (Erg1) channels physically and functionally interact with β1 integrins in many tumor cells including leukemias [149–151]. $K_v11.1$ and β1 integrins modulate each other's function by conformational coupling. Integrin mediated adhesion activates $K_v11.1$ while integrin-signaling such as the tyrosine phosphorylation of the focal adhesion kinase (FAK) requires the activity of $K_v11.1$ [152]. Interestingly, FAK is overexpressed in several invasive tumor cells which might enable them to by-pass integrin signaling and thus survive in the absence of adhesive contacts [153]. The question of whether a “hyperactive” FAK known to form complexes with $K_v2.1$ [154] can – in reverse order – promote integrin-mediated adhesion seems (too) adventurous and needs to remain open. The $Ca^{2+}$ activated $Cl^-$ channel CLCA2 could play a major role in the adhesion of circulating tumor to endothelial cells [155]. However, CLCA2 is expressed at the surface of endothelial cells and, thus, can facilitate the arrest of β4 integrin expressing tumor cells. Store-operated $Ca^{2+}$ entry (SOCE) including the $Ca^{2+}$ release-activated calcium channel protein 1 (Orai1) are required for the extravasation of nasopharyngeal carcinoma cells [156]. Finally, the step of diapedesis could be facilitated by the $Ca^{2+}$-dependent activation of TMEM16A/ANO1. The resulting $Cl^-$ efflux would support local shrinkage of the evading cell and thus help the cell to squeeze its way through the endothelium into the target tissue [68].

## 5. Conclusion

Nearly each single step of the metastatic cascade including migration and invasion depends on the presence or the activity of ion channels and transporters. Different ion channels and transporters as well as aquaporins cooperate in order to generate local pH or $Ca^{2+}$ nanodomains required for adhesion, MMP activity and cytoskeletal dynamics. Others are constituents of focal adhesions, involved in signaling processes or in targeting adhesion molecules. The multitude of channels and transporters contributing to metastasis, and their multifaceted ways of interaction represent an enormous number of targets for anticancer therapeutics. It remains a big challenge to translate the present knowledge on the role of ion transport in tumor cell motility, gained from basic research and preclinical studies, into efficient cancer treatments. While a plethora of studies has demonstrated that a prometastatic role of ion channels and transporters is beyond all question, future studies should deal with questions of (i) how to deliver efficient drugs to poorly vascularized tissues, (ii) how to inhibit ion transport in malignant cells without disturbing healthy cells that express the same transport systems and (iii) how to take advantage of a cocktail containing different drugs at low concentrations that would not affect the transportome of healthy but exclusively of malignant cells.

Compared to the functions of ion channels and transporters in proliferation, motility and invasion, very little is known about their contribution to the adhesion of circulating tumor cells to the endothelium or the actual step of diapedesis under shear stress. It is worth looking into the mechanisms by which ion transporters are involved in adhesion and diapedesis, because during these events they display a better accessibility for a potential drug which then could be administered by intravenous and intralymphatic injection. The efficiency of the lymphatic circulation, however, may represent a limiting factor in distributing the drug.

Targeting the process of EMT is also an option. But it requires the exact knowledge of the differences between regular EMT needed for wound healing, tissue repair and regeneration and that eventually resulting in carcinogenesis.

Thus, future studies aiming at the characterization of the contributions of ion channels and transporters to both EMT and extravasation of tumor cells are indicated.

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